

MARKED UP SPECIFICATION AND CLAIMS

Page 1, line 1, delete the following paragraph:

"This is a continuation of PCT Application No. PCT/EP00/04714, filed 24 May 2000, the entire contents of which is hereby incorporated by reference in this application. This application claims the benefit of U.S. Provisional Application No. 60/138,621, filed 11 June 1999, the entire content of which is hereby incorporated by reference in this application."

and insert the following therefor:

--This application is a continuation of PCT/EP00/04714, filed 24 May 2000, and claims the benefit of U.S. provisional Application No. 60/138,621, filed 11 June 1999, the entire contents of each of which being incorporated herein by reference.—

Page 37, delete the paragraph spanning lines 7-15 and insert the following therefor:

--PCR amplification of the ITS region was performed in a final volume of 100 µl with 20 µl of DNA extracted from the blood samples (for DNA extracted from 5 ml blood samples, 20 µl of a 1/10 dilution is included in the PCR reaction) added to the PCR reaction containing a final concentration 0.25 mM deoxynucleotidetriphosphates (DU/dNTP's[2:1]), 1x reaction buffer (Promega, USA), 3 mM MgCl₂, 1 unit Uracil DNA glycosylase (Longo et al 1990; Roche-Boehringer Mannheim, Germany), 40 pmol each of the forward ITS5 primer (5'-GAAAGTAAAAGTCGTAACAAGG-3') (SEQ ID NO:50) and reverse primer ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (SEQ ID NO:45), 2.5

units of Taq polymerase (Promega, USA), made to a final volume of 100 µl in nuclease free water (Sigma-Aldrich Ltd, UK).--

IN THE CLAIMS

24. (Amended) Method to detect and identify [fungal pathogenic] *Candida* species in a sample, comprising at least the following steps:

- (i) releasing, isolating and/or concentrating the nucleic acids of [the] fungal pathogens possibly present in the sample,
- (ii) if necessary, amplifying the Internal Transcribed Spacer region (ITS) of said nucleic acids with at least one fungal universal primer pair,
- (iii) hybridizing the nucleic acids of step (i) or (ii) with at least one of the following species specific oligonucleotide probes:

TGTCACACCAGATTATTACT (SEQ ID NO:2)

TATCAACTTGTCACACCAGA (SEQ ID NO:3)

GTAGGCCTTCTATATGGG (SEQ ID NO:4),

TGCCAGAGATTAAACTCAAC (SEQ ID NO:5),

GGTTATAACTAAACCAAAC (SEQ ID NO:6),

TTTTCCCTATGAACTACTTC (SEQ ID NO:7),

AGAGCTCGTCTCTCCAGT (SEQ ID NO:8),

GGAATATAGCATATAGTCGA (SEQ ID NO:9),

GAGCTCGGAGAGAGACATC (SEQ ID NO:10),

TAGTGGTATAAGGCGGAGAT (SEQ ID NO:11),

CTAAGGCGGTCTCTGGC (SEQ ID NO:12),

GTTTTGTTCTGGACAACTT (SEQ ID NO:13),
TTGTCACACCAGATTATTACTT (SEQ ID NO:33),
GGTTTATCAACTTGTCACACCAGA (SEQ ID NO:34),
GGTATCAACTTGTCACACCAGATT (SEQ ID NO:35),
GGTTATAACTAAACCAAACCTTTT (SEQ ID NO:36),
GGGAATATAGCATATAGTCGA (SEQ ID NO:37),
GGTTTTGTTCTGGACAACTT (SEQ ID NO:38),

or the RNA equivalents of said probes, wherein T is replaced by U, or the complementary molecules of said probes,

(iv) detecting the hybridization complexes formed in step (iii), and

(v) identifying the [fungal pathogenic] *Candida* species present in said sample, based on the hybridization complex formed.

25. (Amended) Method according to claim 24, wherein the ITS region in step (ii) is limited to the ITS-1 region, and wherein the at least one probe[s] in step (iii) [are] is chosen from the following set of probes:

TGTCACACCAGATTATTACT (SEQ ID NO:2),
TATCAACTTGTCACACCAGA (SEQ ID NO:3),
GTAGGCCTTCTATATGGG (SEQ ID NO:4),
TGCCAGAGATTAACTCAAC (SEQ ID NO:5),
GGTTATAACTAAACCAAAC (SEQ ID NO:6),
TTTTCCCTATGAACTACTTC (SEQ ID NO:7),
AGAGCTCGTCTCTCCAGT (SEQ ID NO:8),

GGAATATAGCATATAGTCGA (SEQ ID NO:9),
GAGCTCGGAGAGAGACATC (SEQ ID NO:10),
GTTTTGTTCTGGACAACTT (SEQ ID NO:13),
TTGTCACACCAGATTATTACTT (SEQ ID NO:33),
GGTTTATCAACTTGTCACACCAGA (SEQ ID NO:34),
GGTATCAACTTGTCACACCAGATT (SEQ ID NO:35),
GGTTATAACTAAACCAAACCTTTT (SEQ ID NO:36),
GGGAATATAGCATATAGTCGA (SEQ ID NO:37),
GGTTTTGTTCTGGACAACTT (SEQ ID NO:38),

or the RNA equivalents of said probes, wherein T is replaced by U, or the complementary nucleic acids of said probes.

28. (Amended) Method according to claim [27] 24 [to detect *Candida albicans* in a sample, said method comprising] wherein the *Candida* species is *Candida albicans* and wherein the at least one probe of step (iii) is

[(i) hybridizing the nucleic acids present in the sample to at least one probe] chosen from among SEQ ID NOs:2, 3, 33, 34 and 35[,

(ii) detecting the hybridization complexes formed, and

(iii) inferring that *C. albicans* is present in said sample, based on the formation of said hybridization complex].

29. (Amended) Method according to claim [27] 24 [to detect *Candida parapsilosis* in a sample, said method comprising] wherein the *Candida* species is *Candida parapsilosis* and wherein the at least one probe of step (iii) is

[(i) hybridizing the nucleic acids present in the sample to at least one probe]
chosen from among SEQ ID NOs:4 and 5[,

(ii) detecting the hybridization complexes formed, and

(iii) inferring that *C. parapsilosis* is present in said sample, based on the
formation of said hybridization complex].

30. (Amended) Method according to claim [27] 24 [to detect *Candida tropicalis* in
a sample, said method comprising] wherein the *Candida* species is *Candida tropicalis*
and wherein the at least one probe of step (iii) is

[(i) hybridizing the nucleic acids present in the sample to at least one probe]
chosen from among SEQ ID NOs:6 and 36[,

(ii) detecting the hybridization complexes formed, and

(iii) inferring that *C. tropicalis* is present in said sample, based on the formation of
said hybridization complex].

31. (Amended) Method according to claim [27] 24 [to detect *Candida kefyr* in a
sample, said method comprising] wherein the *Candida* species is *Candida kefyr* and
wherein the at least one probe of step (iii) is

[(i) hybridizing the nucleic acids present in the sample to at least one probe]
chosen from among SEQ ID NOs:7 and 8[,

(ii) detecting the hybridization complexes formed, and

(iii) inferring that *C. kefyr* is present in said sample, based on the formation of
said hybridization complex].

32. (Amended) Method according to claim [27] 24 [to detect *Candida krusei* in a sample, said method comprising] wherein the *Candida* species is *Candida krusei* and wherein the at least one probe of step (iii) is

[(i) hybridizing the nucleic acids present in the sample to at least one probe] chosen from among SEQ ID NOs:9 and 37[,

(ii) detecting the hybridization complexes formed, and

(iii) inferring that *C. krusei* is present in said sample, based on the formation of said hybridization complex].

33. (Amended) Method according to claim [27] 24 [to detect *Candida glabrata* in a sample, said method comprising] wherein the *Candida* species is *Candida glabrata* and wherein the probe of step (iii) is

[(i) hybridizing the nucleic acids present in the sample to a probe represented by] SEQ ID NO:10[,

(ii) detecting the hybridization complexes formed,

(iii) inferring that *C. glabrata* is present in said sample, based on the formation of said hybridization complex].

34. (Amended) Method according to claim [27] 24 [to detect *Candida dubliniensis* in a sample, said method comprising] wherein the *Candida* species is *Candida dubliniensis* and wherein the at least one probe of step (iii) is

[(i) hybridizing the nucleic acids present in the sample to at least one probe] chosen from among SEQ ID NOs:11, 12, 13 and 38[,

(ii) detecting the hybridization complexes formed, and

(iii) inferring that *C. dubliniensis* is present in said sample, based on the formation of said hybridization complex].

35. (Amended) Method according to [claim] claim 24 wherein the [probes] at least one probe of step (iii) [are] is immobilized to a solid support.

36. (Amended) Method according to claim 24 for the simultaneous detection and differentiation of at least two [fungal pathogenic] Candida species in one single hybridization step, including

(i) releasing, isolating and/or concentrating the nucleic acids of the fungal pathogens possibly present in the sample,

(ii) amplifying the Internal Transcribed Spacer region (ITS) of said nucleic acids with at least one fungal universal primer pair,

(iii) hybridizing the nucleic acids of step (i) or (ii) with at least two of the following species specific oligonucleotide probes:

TGTCACACCAGATTATTACT (SEQ ID NO:2),

TATCAACTTGTCACACCAGA (SEQ ID NO:3),

GTAGGCCTTCTATATGGG (SEQ ID NO:4),

TGCCAGAGATTAAACTCAAC (SEQ ID NO:5),

GGTTATAACTAAACCAAAC (SEQ ID NO:6),

TTTTCCCTATGAACTACTTC (SEQ ID NO:7),

AGAGCTCGTCTCTCCAGT (SEQ ID NO:8),

GGAATATAGCATATAGTCGA (SEQ ID NO:9),

GAGCTCGGAGAGAGACATC (SEQ ID NO:10),

TAGTGGTATAAGGCGGAGAT (SEQ ID NO:11),
CTAAGGCGGTCTCTGGC (SEQ ID NO:12),
GTTTTGTTCTGGACAACTT (SEQ ID NO:13),
TTGTCACACCAGATTATTACTT (SEQ ID NO:33),
GGTTTATCAACTTGTTCACACCAGA (SEQ ID NO:34),
GGTATCAACTTGTTCACACCAGATT (SEQ ID NO:35),
GGTTATAACTAAACCAAACCTTTTT (SEQ ID NO:36),
GGGAATATAGCATATAGTCGA (SEQ ID NO:37),
GGTTTTGTTCTGGACAACTT (SEQ ID NO:38),

or the RNA equivalents of said probes, wherein T is replaced by U, or the complementary molecules of said probes,

wherein said probes have been immobilized to a solid support on specific locations,

(iv) detecting the hybridization complexes formed in step (iii),

(v) identifying the species present in the sample by the location of the hybridization signal on the solid support.

37. (Amended) Isolated oligonucleotide molecule [having] consisting of a nucleotide sequence represented by any of SEQ ID NOs:2 to 13 or 33 to 38, or the RNA equivalents of said SEQ IDs wherein T is replaced by U, or the complementary nucleic acid of said SEQ IDs.

38. (Amended) Isolated oligonucleotide molecule according to claim 37, for use as a species specific primer or probe in the detection of one of the following fungal

pathogens: *Candida albicans*, *Candida parapsilosis*, *Candida tropicalis*, *Candida kefyr*, *Candida krusei*, *Candida glabrata*, and *Candida dubliniensis*], *Aspergillus flavus*, *Aspergillus versicolor*, *Aspergillus nidulans*, *Aspergillus fumigatus*, *Cryptococcus neoformans* and *Pneumocystis carinii*].

40. (Amended) [Method according to claim 24, wherein the] Method to detect and identify *Candida* species in a sample, comprising at least the following steps:

(i) releasing, isolating and/or concentrating the nucleic acids of fungal pathogens possibly present in the sample,

(ii) if necessary, amplifying the Internal Transcribed Spacer region (ITS) of said nucleic acids with at least one fungal universal primer pair,

(iii) hybridizing the nucleic acids of step (i) or (ii) with at least one of the following species specific oligonucleotide probes:

TGTCACACCAGATTATTACT (SEQ ID NO:2)

TATCAACTTGTCACACCAGA (SEQ ID NO:3)

GTAGGCCTTCTATATGGG (SEQ ID NO:4),

TGCCAGAGATTAAACTCAAC (SEQ ID NO:5),

GGTTATAACTAAACCAAAC (SEQ ID NO:6),

TTTTCCCTATGAACTACTTC (SEQ ID NO:7),

AGAGCTCGTCTCTCCAGT (SEQ ID NO:8),

GGAATATAGCATATAGTCGA (SEQ ID NO:9),

GAGCTCGGAGAGAGACATC (SEQ ID NO:10),

TAGTGGTATAAGGCGGAGAT (SEQ ID NO:11),

CTAAGGCGGTCTCTGGC (SEQ ID NO:12).

GTTTTGTTCTGGACAACTT (SEQ ID NO:13).

TTGTCACACCAGATTATTACTT (SEQ ID NO:33).

GGTTTATCAACTTGTCACACCAGA (SEQ ID NO:34).

GGTATCAACTTGTCACACCAGATT (SEQ ID NO:35).

GGTTATAACTAAACCAAACCTTTTT (SEQ ID NO:36).

GGGAATATAGCATATAGTCGA (SEQ ID NO:37).

GGTTTTGTTCTGGACAACTT (SEQ ID NO:38).

or the RNA equivalents of said probes, wherein T is replaced by U, or the complementary molecules of said probes,

(iv) detecting the hybridization complexes formed in step (iii), and
(v) identifying the *Candida* species present in said sample, based on the hybridization complex formed;

said oligonucleotide probes [comprise] including a homopolymer tail which is added at the 3' or 5' extremity of the probe.